

The University of Chicago

**EXPERIMENTAL CHANGES IN END-FEET
OF HELD-AUERBACH IN THE
SPINAL CORD OF THE CAT**

AN EXPANSION OF A DISSERTATION SUB-
MITTED TO THE FACULTY OF THE DIVISION
OF THE BIOLOGICAL SCIENCES IN CANDIDACY
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1937

By
RUTH I. BARNARD

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TWELVE TEXT FIGURES AND ONE PLATE (TWO FIGURES)

Two papers on the boutons terminaux² were published by Hoff in 1932, reporting changes which these boutons undergo when the axons or cell bodies of which they are presumably the nerve endings were injured or destroyed. He described experiments on cats in which the motor cortex was ablated or in which the dorsal roots of the spinal nerves were cut. His reports contain the following statements:

1. The end-feet are "small, oblong loops, occurring at the end of nerve fibers and applied to cell bodies and their dendrites."
2. They vary in size from 2.2 by 1.1 μ to 1 by 0.5 μ .
3. All the end-feet on a particular cell are stained by the procedure he used.
4. The total number of boutons on any one cell varies, in the same and comparable preparations.
5. There is no continuity between end-feet and intracellular neurofibrils.

¹ This work is an expansion of a thesis submitted in partial fulfillment of the requirements for a Ph.D. degree. It was aided by grants from the Dr. Wallace C., and Clara A. Abbott Memorial Fund of The University of Chicago, and from the Rockefeller Foundation.

² Bouton terminale, end-foot of Held-Auerbach, and end-bulb are the three terms in most common use in the recent literature to designate the small axon terminations on the cell bodies and dendrites of the spinal cord.

6. Experimental changes were observed in the boutons on the intermediate cells of the spinal cord upon cutting the axons of the pyramidal tract of which they were, presumably, the terminals. Boutons became larger, more granular, the formerly clear loop becoming filled in, and by the end of the sixth day all the degenerated boutons have disappeared.

7. This degeneration does not extend across the synapse into the cell of termination.

Hoff demonstrated the boutons by means of the Cajal reduced silver technique, modification 6a, after perfusing 75 cc. of 10% chloral hydrate per kilogram of body weight through the abdominal aorta of the anaesthetized animal.

The essential details of this work were confirmed by Foerster, Gagel and Sheehan ('33) on material in which the posterior roots of rhesus monkeys were cut. They also found progressive degenerative changes in the end-feet in which the posterior root fibers terminated, corresponding to those found by Hoff. Gibson ('37) reports such changes also, and describes in great detail the changes which occur at the various time periods, up to 120 hours postoperatively. "An average degenerating bouton passes through the process of hypertrophy, elongation, fragmentation and granulation, and from the circular to the ellipsoid and back to the circular again." In his diagrammatic figure he pictures the following stages:

Normal: circular, clear loop, 2 by 2 μ .

24 hours: circular, thick loop, 2.4 by 2.4 μ .

48 hours: irregularly circular solid body, 2.8 by 2.8 μ .

72 hours: irregularly ellipsoid body, 4.1 by 2.04 μ .

96 hours: irregularly ellipsoid solid body, 4.5 by 2.4 μ .

120 hours (a): irregularly ellipsoid solid body, 4.8 by 2.8 μ .

120 hours (b): irregularly circular, solid, with slight fragmentation, 4.5 by 2.8 μ .

120 hours (c): irregularly circular, solid, with greater fragmentation, 4.1 by 4.1 μ .

120 hours (d): granulation.

These stages may be compared with those found by Hoff.

	HOFF	GIBSON
Normal	Oblong, clear, 2.2 by 1.1 μ .	Circular, clear 2 by 2 μ .
24 hours	Swelling of loop, thick, slightly granular, stained darkly. Small fibril with moniliform swelling up the center of the loop.	Circular thick loop. 2.4 by 2.4 μ . No fibril mentioned. Uniform swelling, loop-like appearance gradually lost.
48 hours	Darkly staining. Some entirely opaque, others lighter in the center, with tiny fibril. More pronounced irregularity and granulation, variation in shape. Round, pear-shaped, greatly elongated. Increase in size.	Central hollow lost. Circular configuration gone. Irregular varicosities. Irregularly circular, 2.8 by 2.8 μ .
72 hours	Swollen, elongated, granular. Loop scarcely distinguishable. Variable shape, mostly long and spatulate or pear-shaped, and thick. 4.5 by 1.5 μ to 6.1 by 2.3 μ .	Noticeable increase in length and area. Affords unmistakable evidence of degeneration. (No details given.) Picture shows irregular ellipsoid solid body 4.1 by 2.04 μ .
96 hours	Merely granular mass.	Granular. Increased length and width. Smooth contour interrupted by large granules ready to break away. Has lost shape of intact bouton. 4.5 by 2.4 μ .
120 hours		Increased fragmentation of formerly solid black mass. Tendency toward circular shape again. Increased diameter attributed partly to interstices between segments. 4.8 by 2.8 μ to 4.1 by 4.1 μ . Granulation.
144 hours	All degenerated boutons gone.	

Lawrentjew ('34) described the degeneration and disappearance of end-feet in sympathetic ganglia when preganglionic fibers were cut.

These findings of Hoff, Foerster, Gagel and Sheehan, Lawrentjew, and Gibson are of great importance in fiber tract neuroanatomy, because there has been no method prior to these reports of determining the exact termination of a fiber tract. We must first find out, however, how constant are the

results to be obtained by the methods used by these workers, under differing conditions of experiment and procedure.

Since the reports described above were published, three other reports have cast some doubt upon the uniformity of result to be expected by silver impregnation methods which may make conclusions difficult as to whether the termination of a fiber bundle can be accurately determined or not, by a demonstration of changes in the terminal end-feet or boutons terminaux.

Barr ('39) studied the end-foot synapses in the spinal cord of normal cats. He used modification 4 of Cajal's reduced silver method because he found that only a small percentage of the end-bulbs stained with the modification 6a of Cajal's method. He found considerable variation in the staining of the end-feet from cell to cell even in the same section. He found end-feet varying in size from $0.5 \times 1.0 \mu$ to $4.0 \times 5.0 \mu$. The small endings are well defined rings but may be opaque or have a fine fibril passing down the center of the ending. The largest endings may be round, oval or spatulate, are usually opaque, and may present a granular or reticulated appearance.

Schimert ('38) attempted to find the mode of ending of the vestibulo-spinal tract in the cat. He destroyed the vestibular nuclei with a thermo-cautery and killed the animals 7 to 10 days after operation. The Cajal method, according to Schimert, is not suited to this line of investigation because the end-feet in the normal animal often show considerable variation, very large club endings often occurring which are two or three times the size of the smaller ones. He used a Bielschowsky method and claimed that the only good criterion for designating end-feet as degenerating is a varicose thickening of the fibers as well as of the end-feet with vacuolation to make a characteristic foamy appearance. Schimert stated further that a large number of end-feet are so completely impregnated with silver as to appear completely black, even in the normal animal.

Phalen and Davenport ('37), using a Cajal method as modified by Davenport ('33), studied the end-bulbs in a number of different vertebrates. They removed the tissue within $\frac{1}{2}$ hour after the death of the animal and fixed by immersion. They state, "The marked variation in size and shape of the bulbs and their variable abundance on the ganglion cell or its dendrites indicate that their degeneration would not be a suitable index for locating synaptic connections of degenerating tracts unless such degeneration were massive in its extent."

In view of these contradictory reports in the literature, the experiments to be described and discussed below were performed to determine (1) the nature and lability of the end-foot type of synapse in the spinal cord, (2) its reaction to injury of the nerve cell, and (3) its relations and connections with both the cell of origin and cell of termination.

It was considered essential to use the same animal (cat) and to attempt to repeat some of the previous experiments reported in the literature as closely as possible, because other types of synapses may not be comparable to the end-foot synapse in the spinal cord. Studying another type of synapse, the mossy endings of the brachium pontis fibers in the cerebellum, Snider ('36) found very definite degenerative changes in the mossy endings after the brachium pontis was cut. His pictures show significant differences in the operated material from normal material, the mossy terminals undergoing swelling and granular degeneration when the brachium pontis was sectioned. He also used the Cajal method and found that there was no difference in the demonstrations of mossy terminals obtained whether he fixed by perfusion or immersion. Snider repeated this work in our laboratory in 1938, confirming his previous results. The observations reported in this paper are to be considered as applying only to the end-foot synapse in the spinal cord of cats.

METHODS AND DESCRIPTION OF NORMAL MATERIAL

In order to get material comparable to that reported by others who have studied the end-foot experimentally, the

Cajal reduced silver method was used for the greater part of the microscopic preparations. Davenport ('33) has reported that hydroquinine, as used in the Cajal 6a modification, gives a spotty, coarsely granular appearance to the impregnated block of tissue, and that pyrogallie acid is preferable. I have found this true, and have therefore used the method recommended by Davenport.

The animal was killed by illuminating gas, and the thorax opened immediately upon cessation of respiration, usually while the heart was still beating. A cut was made in the wall of the left ventricle, and a cannula inserted through it and tied into the ascending aorta. The right auricle was opened to permit the escape of fluid before reaching the lungs. From 350 to 500 cc. of 10% chloral hydrate were perfused from a height of 6 feet and the bottle then lowered to 4 feet and another 350 to 500 cc. perfused without interruption, making a total of 800 to 1000 cc. It will be noted that this is about four times as much perfusate as used by Hoff, who perfused only 75 cc. per kilogram of body weight in a cat of an average weight of 2 kg. His technique differed also, in that he perfused upward into the abdominal aorta, allowing escape of fluid through the inferior vena cava. After perfusion through the ascending aorta the spinal cord showed no vessels grossly, and the microscopic sections showed the capillaries and larger vessels to be free of blood, and usually dilated.

After perfusion was completed, appropriate levels of the spinal cord were cut into small (5 to 10 mm.) blocks, and immersed in 10% pyridin for 24 hours. The blocks were then washed thoroughly in many changes of distilled water for $\frac{1}{2}$ hour, and immersed in 1.5% silver nitrate, where they remained at 37°C. for 6 days. They were washed again in many changes of distilled water for $\frac{1}{2}$ hour, and reduced in 2% pyrogallie acid for 5 hours. They were then washed again, dehydrated rapidly, and embedded in paraffin.

It has been found, in studying normal material prepared by this and other reduced silver methods (Phalen and Davenport, '37) that silver impregnation and reduction, either with

slight variations or no differences in technique, give widely differing appearances. In a lightly impregnated block of tissue fewer end-feet are seen, and most of these appear as thin rimmed loops with clear centers. In an overstained block, on the other hand, more end-feet are stained, and they appear larger, thicker, and frequently entirely solid. In the well-impregnated block, intermediate between these two extremes, boutons of both types are found in the normal animal. These vary in size from 0.5 by $0.5\ \mu$ to 3 by $5\ \mu$. Barr ('39) says, "End-bulbs of widely different size and shape may be present within a small area on the same cell." Such variations due to the technique applied (Phalen and Davenport, '37) make variations due to the experimental procedure difficult to detect, unless these latter are massive and highly specific.

To obviate this difficulty as much as possible, staining of end-feet with histological dyes was carried out on many experiments along with the Cajal impregnation. The fixation of the tissues offers the greatest problem. The purpose of fixation is to preserve the tissue for study in a condition as closely approximating that of the living as possible. This means fixation must be prompt, in order to avoid postmortem changes. Since penetration of a block of tissue by the fixative is not immediate, it is believed that perfusion of the fixing fluid through the blood vessels during life or immediately after death, is highly desirable. In addition to insuring promptness of fixation, it is preferable to avoid undue swelling or shrinkage of the tissues, which would alter the true appearance of the structures under study, as well as the relations of the various constituents to each other. The fixative must obviously not be incompatible with the stain or metallic impregnation used.

If fixation be adequate (rapid enough, with minimum distortion), end-feet may be demonstrated with many of the ordinary histological stains, as aniline acid fuchsin-methyl green, Mallory's connective tissue stain, Mallory-azan, haematoxylin and eosin, haematoxylin-eosin-azure, and iron haematoxylin. The "histological" technique, i.e., staining of sections

with histological dyes (in contrast to "impregnation" technique, or impregnation of a block of tissue with a metal), used routinely in this work, and arrived at after many trials, was perfusion with Regaud's formol-bichromate, and staining with either aniline acid fuchsin-methyl green, or Mallory's connective tissue stain. The latter gave slightly better differentiation of the end-feet from the surrounding tissue. Perfusion was carried out as with the chloral hydrate.

The normal appearance of end-feet in Cajal material (fig. 12) is that of round, elliptical, or elongated masses, of extremely variable size, either as loops, or solid masses, or reticulated or granular structures intermediate between the two extremes. They may be attached to an axon or broken off from it. They may be applied to a cell body or its dendrites, or may be separated from it. Examples of all of these conditions may be found in any one preparation. The total number of boutons on a cell varies from just a few to a hundred or more. There is no indication that all the boutons are visualized in any preparation.

Although Hoff says the method gives "consistent and equable impregnation in which all the boutons terminaux and boutons de passage are stained," Gibson modifies this somewhat by stating: "It should be stated clearly that the bouton method is a silver method and is subject to the vagaries of all types of silver staining In most cases it is impossible to stain more than a small proportion of the degenerated boutons around a cell, but those which do appear are usually well defined." Gibson makes no statement as to the completeness of impregnation of normal boutons.

Barr ('39) describes large end-bulbs of the order of 4.5 by 5.0 μ frequently occurring within a distance of several microns from small endings less than 1.0 μ in diameter. He says, "The occasional occurrence of large endings should not be interpreted as indicative of bouton degeneration as a result of cutting the apparent axons since such boutons occur in the normal cord." He also states that methods using chloral hydrate as the primary fixative result in the staining of only a

small percentage of the end-bulbs. With formalin fixation he was able to get a much larger percentage of the end-feet to stain.

In histologically stained material the end-feet almost invariably appear round, rarely elongated, solid, somewhat granular bodies, taking the axis cylinder and mitochondrial stains. Occasionally a less intensely stained center is seen, but true loops are extremely rare. They are nearly all of the same size, and measure approximately 1μ in diameter. As in the Cajal method, end-feet may or may not appear attached to fibers. With Regaud fixation there is little shrinkage of the cell body, and end-feet are usually applied to it, rather than being pulled away from it. In both the Regaud and the Cajal material end-feet were more easily demonstrated on the dendrites than on the cell body itself, probably because dendrites are more apt to be cut tangentially, thus permitting one to see more end-feet than on the cross-cut cell body. Differentiation in the stained preparations, however, is not marked, the end-feet appearing as pink bodies on a lighter pink or lavender cell body or dendrite. In this material, as in the Cajal, one has no assurance that all the end-feet are stained. This is indicated by the variation one sees in the number of end-feet from cell to cell, and from preparation to preparation, and also by the fact that if one counterstains a silver preparation with a histological stain, more end-feet can be seen than were originally visible by impregnation alone. It is impossible to know how many more are still not visualized. Even in a relatively thick section, there are many pieces of dendrite that cannot be followed to their source, and even though they are covered with end-feet, cannot be included in counts limited to cell bodies.

EXPERIMENTS AND OBSERVATIONS

Section of axis cylinders

Following as closely as possible the pyramidal tract experiment of Hoff, in order to determine the usefulness of the method of bouton degeneration in determining the endings of

fiber tracts, I destroyed by electrocautery, the entire cruciate gyrus, unilaterally, in five cats. The animals were sacrificed 3, 4, 5, 6, and 7 days later, and the cervical and lumbar segments of the spinal cords were prepared according to the Cajal method, as described above.

The lesion was confirmed by identification of the cruciate gyrus and observation of the gross lesion at autopsy, and also by the behavior of the animal during the interval between operation and sacrifice. There was weakness of the limbs of the opposite side, and diminution or loss of position sense. This may be tested by placing the cat along the edge of a table and allowing the front and hind limbs of one side to hang over the edge of the table. On the normal side, the cat does not maintain that position, but retracts the legs immediately. On the other side, the legs remain in this position until the animal gets restless and starts to move away altogether. With such a lesion, also, the cat will walk on the dorsum of the paw, both by force and spontaneously, while normally it will not permit this position even momentarily.

The end feet on the cells of the intermediate column and on the anterior horn cells were studied in all cases. Since no one working on this problem has given boundaries of the so-called intermediate column, it is necessary that I identify those cells which are designated as such. The cells which lie ventral to the dorsal horn and dorsal to a line extended laterally from the anterior commissure, exclusive of the large Clark's column cells and the delicate tapering intermedio-lateral column cells are those which I designate as intermediate column cells. Although Hoff does not define them, it will be seen by reference to his line drawings that these are the cells which he also calls intermediate. This definition corresponds to Cajal's designation of the intermediate gray nucleus. The cells ventral to this are of course anterior horn cells.

End-feet were classified as group I: small, round, clear loops, corresponding to Hoff's normal end-feet, and group II, large, round or elongated, either solid or granular end-feet, corresponding to Hoff's criteria for degenerating end-feet.

In a normal animal, out of 134 end-feet on two intermediate cells of the cervical cord, forty-two belonged to group II according to the criteria outlined by Hoff. In the animal sacrificed 3 days after destruction of the right cruciate gyrus, four intermediate cells on the right side of the cervical enlargement showed 76 group II boutons, and 84 of group I, and on the left side of the cord four cells showed 71 group II and 155 group I. Figures 1 and 2, of anterior horn cells from opposite sides of the same section, show both types of terminals similar to those on the intermediate cells. In Hoff's material the crossed side was "peppered" with degenerating terminals at 72 hours, and on the uncrossed side an occasional cell shows them, although he cites no actual figures. Cells of the intermediate column were studied in both cases.

Five days after operation, counts on two anterior horn cells of the lower cervical cord showed 76 group II to 162 group I boutons on the uncrossed side, and 57 group II to 175 group I on the crossed side. Seven days after operation Hoff states that all the degenerated boutons have disappeared, leaving noticeably fewer on the operated side. In our material on five cells picked at random from the intermediate column of each side of the cord, after 7 days, the boutons totaled 229 on one side, and 236 on the other. As in all the other sections, some were group I and some were group II. In this material, an occasional bouton was seen which was larger than 3 by 5 μ , the largest seen in the normal, but with a few such exceptions, the boutons all fell within the wide limits of normal variation, as to size, shape, density of staining, and frequency.

Since fibers of many sources end around the cells of the spinal cord, it is probable that only a few end-feet on any one cell come from the same source, such as the cells of the cruciate gyrus. In this case, it might be difficult to detect degenerative changes in a few boutons among a multitude of normal ones, whereas if the lesion involved more fiber tracts, changes in end-feet would be more obvious, even though there were wide variation among the normal boutons.

To determine if such were the case, a series of cats was subjected to hemisection of the midthoracic cord, plus section of two to four dorsal roots at and below the level of section, the cord being exposed by the dorsal approach, under aseptic



All figures were outlined with a camera lucida, using a Spencer 2 mm. apochromat and 10 $\times$ oculars. The original magnification was 1440 diameters, reduced to $\times$ 960. In figures 1-12 the end-feet were drawn as accurately as possible, the cells and dendrites, nuclei and neurofibrillae merely indicated diagrammatically.

Fig. 1 Anterior horn cell, lower cervical, left side, 72 hours after ablation of the entire right motor cortex.

surgical technique. The lesion was tested physiologically by symptoms of paralysis and loss of sensation of the lower limbs, and confirmed grossly at autopsy. The animals were sacrificed as previously described after 2, 3, 4, 6, 14, and 36 days, and spinal cord from immediately below the level of

the lesion was prepared for microscopic study. Each was run in duplicate, and one animal of each pair perfused with chloral hydrate for Cajal impregnation, the other with Regaud, for staining.

None of the twelve cats showed any differences in the end-feet of the cells of the two sides of the spinal cord, or any differences from the normal, in the Cajal material. From 2 to



Fig. 2 Anterior horn cell, lower cervical, right side, from same section as figure 1. In this section there are considerable numbers of large end-feet, some of which have thick rims and some completely blackened. A few are granular.

6 days postoperatively, end-feet varied in size, shape, and density of staining as did the normal. In the animals sacrificed after 14 and 36 days the numbers of end-feet on the two sides were comparable, both to each other, and to the normal, cells on both sides of the spinal cord showing wide variation in the numbers of end-feet on them. End-feet varied from



Figures 3 to 10 are all from low thoracic segments of the same spinal cord from a cat sacrificed 3 days after hemisection of the left side of the midthoracic cord.

Fig. 3 Right anterior horn cell, low thoracic segment, 3 days after hemisection of the midthoracic cord.

small, clear, round loops, to large elliptical, solid and reticulated bodies in all sections, and in all cell groups of both sides of the spinal cord below the level of section (see figs. 3 to 11).

End-feet on all of the cells of the intermediate column and anterior horn were counted in thirty-eight serial sections of 15μ each in the spinal cord of an animal which was killed



Fig. 4 Left anterior horn cell from the same section as figure 3.

3 days after a right midthoracic hemisection, the sections being from just below the level of section.

The left side of the cord was more darkly impregnated than the right, which was the side of the hemisection. From the

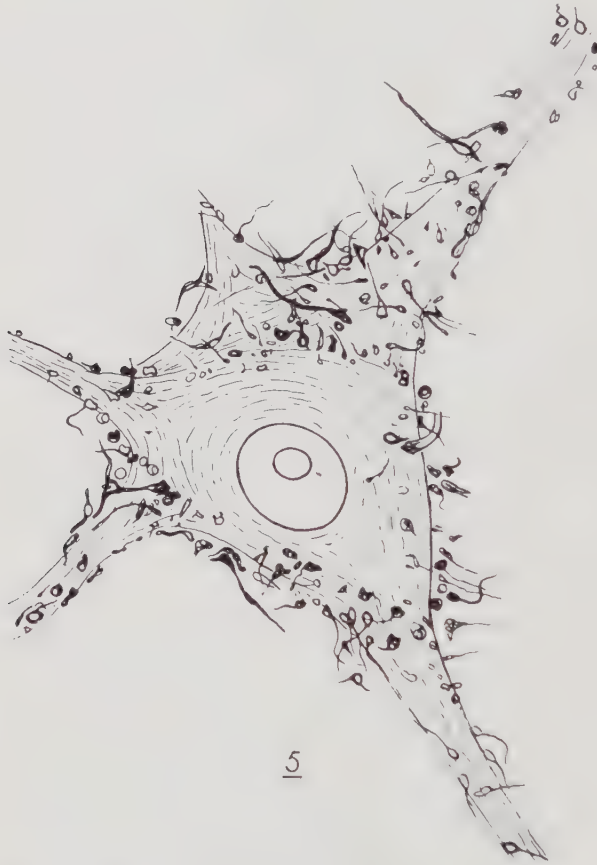


Fig. 5 Right anterior horn cell from the same cord as figures 3 and 4, 250μ below section from which 3 and 4 were drawn. Right side.

figures it will be seen that there were more end-feet bilaterally on anterior horn cells than on cells of the intermediate column. The total number of end-feet on intermediate cells was 4486 on the right, 5126 on the left, making 9612 in all. On the

anterior horn cells there were 7463 end-feet on the right side, 7017 on the left, or 14,480 in all.

END-FEET	RIGHT SIDE		LEFT SIDE	
	Group I	Group II	Group I	Group II
Intermediate cells	2487	1999	2590	2536
Anterior horn cell	4455	3008	3612	3405
Total number of end-feet	6942	5007	6202	5941

From these figures it can be seen that there are more group II end-feet on the left side of the cord than on the right, both actually and relatively to the total. This is exactly opposite to what might be expected on the basis of Hoff's report in the right-sided hemisection at the height of the reaction.

In all of the spinal cords fixed with Regaud and stained with aniline acid fuchsin, all of the boutons on both sides of



Fig. 6 Anterior horn cell, left side of same section as figure 5.

the spinal cord were very close to the same size, small, round, granular, and of similar density, just as is found in the normal animal, and in contrast to the variations found in the Cajal impregnated spinal cords of normal and operated animals.



Figures 7 to 10 taken from a section $200\ \mu$ below the section from which figures 5 and 6 were drawn.

Fig. 7 Right anterior horn cell, low thoracic, 3 days after left midthoracic hemisection.

Ventral root section

In view of the observation that cutting fiber tracts caused no demonstrable changes in the end-feet in which they terminated, and in order to determine the intimacy of the relationship between the end-foot and the cell of termination, ventral roots of the spinal cords of cats were sectioned. Three adjacent ventral roots between C6 and T2 were sectioned intradurally and unilaterally in twenty-eight cats, and the animals killed after 2, 3, 4, 5, 6, 10 and 14 days. In some of these animals dorsal roots were also unavoidably cut. There were four animals in each group, two of which were perfused with

chloral hydrate for Cajal impregnation, and two of which were perfused with Regaud's fluid and the sections stained with Mallory's connective tissue stain.

In the Cajal material the chromatolyzed cells in the earlier stages are somewhat swollen, with irregular, eccentric, sometimes pyknotic nuclei. The perinuclear region is a homogeneous, muddy brown or yellow, devoid of neurofibrils. These



Fig. 8 Left anterior horn cell 3 days after left midthoracic hemisection.

appear to be pushed out and concentrated at the periphery of the cell, as a dense, black, wavy line. Normal cells show regular, central nuclei, with well impregnated perinuclear and peripheral neurofibrils. The end-feet on the nonchromatolyzed cells of both sides of the spinal cord appear as on other normal cells, with the same wide variations in size, shape, number and density of impregnation.

The end-feet on the chromatolyzed cells show changes in a measure comparable to, but by no means identical with those

described by Hoff and Gibson after section of afferent fibers. Two days after ventral root section silver impregnated sections show a breaking up of the continuous membrane of the end-feet, which have partially disintegrated, so as to form irregular granules, crescents, and fragments. Some of these are attached to nerve fibers, making their identification as end-feet unquestionable. No enlargement, or change in shape



Fig. 9 Right anterior horn cell, low thoracic, 3 days after left midthoracic hemisection.

from circular to ellipsoid was seen, apart from the normal variations already described.

At 3 days this fragmentation has progressed, involving more end-feet. The difference seen in the end-feet in the various periods of time after nerve section is entirely one of quantity, and no qualitative differences were observed to be present. In addition to the increase in the number of frag-

mented end-feet on the chromatolyzed cells, these cells showed a decrease in the total number of end-feet present, as compared with nonchromatolyzed cells from the opposite side of the spinal cord. A count of five cells totalled 61 boutons on the operated side, 199 on the normal side. These changes are most marked on the cell bodies, the end-feet on the dendrites for the most part showing no change.



Fig. 10 Left anterior horn cell, low thoracic, 3 days after left midthoracic hemisection.

The fragmentation is still more marked at 4 days (fig. 13), and by 5 days end-feet on the chromatolyzed cells are almost entirely absent, and fragmentation correspondingly less in evidence. At 6, 7, and 10 days the picture is very striking (fig.



Fig. 11 Left anterior horn cell, low thoracic segment, 36 days after left mid-thoracic hemisection. There are sixty-nine end-feet, of which nine are large, dark-staining or granular.

14). There are no end-feet to be seen on the bodies of chromatolyzed cells, which are remarkably homogeneous in their appearance. The dendrites of these cells show a few end-feet. The normal cells in these same preparations have numerous end-feet on both the cell bodies and dendrites.

At 14 days no fragmentation of end-feet was seen, but end-feet were present on chromatolyzed cells, although not in as large numbers as on the normal cells. Whether this is to be interpreted as a real change occurring between 10 and 14 days, or merely an accident of this series, cannot at present be said with certainty.



12

Fig. 12 Intermediate column cell from a lumbar segment of the spinal cord of a normal cat. There are forty end-feet, of which seven are large and either solid or reticulated.

The Regaud material, stained with Mallory's stain, is confirmatory of the Cajal material, although the end-feet are not as well differentiated from the rest of the structures in the vicinity. Early fragmentation of end-feet on chromatolyzed cells is seen, similar to, but not as striking as that seen in the Cajal material. The progressive disappearance of end-feet on chromatolyzed cells is readily appreciated, however. At 3 days, a five cell count totalled twenty-seven on the operated side, seventy on the normal. On no section is there any apparent change in other elements of the spinal cord.

Postmortem changes in end-feet

Six cats were killed with illuminating gas and allowed to remain untouched for 10, 25, or 40 minutes before being perfused with chloral hydrate. The spinal cords were then impregnated with silver. The sections of spinal cord from the animals perfused 10 minutes after death cannot be distinguished from those perfused during life, or within a few seconds of death. At the end of 25 minutes, however, fragmentation of end-feet is very marked, the cells showing a few typical end-feet, but being liberally covered with granules and crescents, some of which retain their axis cylinder connections. At 40 minutes, practically no intact end-feet remain, but granules and debris are present.

DISCUSSION

It has been seen that there are many discrepancies between the observations made in the course of these experiments, and those made by Hoff, Gibson, and others on comparable material.

One of the first problems which presents itself is the adequacy of the methods used for the demonstration of end-feet. Although Hoff says the Cajal method gives constant impregnation of all end-feet, Gibon says that the method is subject to the vagaries of all types of silver staining and that in most instances "it is impossible to stain more than a small proportion of the degenerated boutons around a cell." He makes no statement, however, as to the percentage of normal boutons stained in any one preparation, nor as to what this proportion of stained degenerating boutons is and how one could determine the total number of boutons.

It has been noted in this material, as well as in that of Phalen and Davenport, that an appreciable number of boutons are found in the normal animal which correspond to those described by Hoff and Gibson as degenerating. Now if it is true, as it appears to be, that only a fraction of the total number of boutons are stained, and that they are of variable form, even in the normal, it becomes difficult to appreciate small

changes in the appearance and distribution of the end-feet in the experimental animals. For example, suppose the pyramidal fibers of a cat have been destroyed, and 3 days later the animal is killed and Cajal sections of the spinal cord prepared. A certain number of "degenerating" boutons and a certain number of "normal" ones can be counted on each cell of the spinal cord. How is one to determine the actual number of normal and degenerating boutons and the existence of an absolute or relative increase in degenerating end-feet in an incompletely stained spinal cord, as compared with an incompletely stained normal spinal cord? Even admitting, for the moment, the truth of Gibson's statement "those (degenerated boutons) which do appear are usually well defined," this is of little value, because of necessity, this is a problem of comparative, not absolute change.

For the purpose of determining quantitative changes in tissues, complete impregnation is essential. There is evidence, seen in the counterstained preparations, that the Cajal method is inadequate on this score. For the purpose of studying qualitative changes in such minute structures, a method must be used which preserves these structures in a condition which approximates the living as closely as possible, and which allows of good visualization of the structures in fixed and stained preparations. It is believed that neither the Cajal nor the staining method is adequate from the qualitative viewpoint. Depending on the depth of impregnation, which cannot be absolutely controlled, different appearances of the synapse are obtained. Histological staining gives poor differentiation of the end-feet from other structures in the vicinity, and careful detailed study is therefore not possible. The appearances obtained from dye-stained and silver impregnated preparations are quite different, the latter being more distinct, the former more uniform. Which is one to select as the true appearance, for use in comparison with experimental material?

In accordance with the generally accepted statement that the part of a cell separated from its nucleus dies, it is logical,

as Gibson says, "to suppose that boutons terminaux undergo the changes described whenever their axon is interrupted." He goes on to say, "It would be unfair, however, to assume that because abnormal boutons were not stained by the silver technique, no degenerative changes had taken place." On the other hand, it would be just as unfair to assume, on the basis of incompletely stained material, that such degenerative changes had taken place, and that they were specific and quantitative. Proof, because of the vagaries of the method, would be lacking, regardless of what one would logically expect to find.

The following observations were made in the experimental material:

1. In normal animals, extremely variable numbers of end-feet are found on the cells of the spinal cord, with no assurance at any time that all, or even a majority of them are ever stained.

2. In normal animals there is wide variation in size, shape, and density of impregnation of the boutons.

3. After neither motor cortex ablation nor hemisection of the spinal cord, were differences from the normal observed.

What explanations can be offered for this disagreement with other reports in the literature? It has been noted that fixation of the tissue is important in visualizing end-feet. Bartelmez and Hoerr ('33) found total disappearance of nerve endings in the central nervous system because of fixation by immersion. Perfusion of the fixative, it has been said above, probably more nearly fulfills the requisites of good fixation than does fixation by immersion, because of more prompt action of the fixative. However, to ensure good fixation, even by perfusion, the blood should be washed out with the fixative, and the quantity of fluid used must be adequate. Spinal cord at best is difficult to perfuse, and 150 cc. of fluid would produce only a slight effect on the spinal cord. The rest of the fixation occurring after immersion, postmortem changes would meanwhile continue. End-feet which have already been damaged by cutting their axons might show more extensive postmortem

changes than normal end-feet, whereas in more adequately fixed material the two would be more similar in appearance, and the abnormal end-feet would therefore fall within the wide limits of normal variability already outlined.

On the Regaud material, boutons are all about the same size, as Hoff found in his Cajal material, and about 1μ in diameter. They also are not as numerous as one would expect them to be, and no differences between the normal and experimental could be demonstrated. On the basis of this and the Cajal material it is difficult to say whether no changes take place in end-feet when they are severed from their cells of origin, or whether such changes do occur, but because of the inadequacies of the methods available at the present time, they cannot be demonstrated with sufficient certainty and consistency to be of value in experimental neuroanatomy.

In experiments in which ventral roots were sectioned, in contrast to those just discussed, degenerative changes were seen in the end-feet on the injured cells. These changes consisted in fragmentation of the end-feet, but the minute and detailed changes described by Hoff and Gibson were not seen. Fragmentation was not progressive; that is, an end-foot was either fragmented or it was not, and stages of fragmentation could not be followed. It is easy to see why we are able to demonstrate degenerative changes in end-feet when ventral horn cells are injured, but were unable to do so when the injury was to the cell of origin. In the former instance, degeneration was massive, and all the end-feet on a particular cell were involved. But it is not so easy to interpret these findings and say why end-feet should undergo these changes under such circumstances. Several alternate possibilities present themselves:

1. The end-foot is dependent upon its cell of termination for its morphological integrity, and undergoes degeneration when the terminal cell is injured. This may be on the basis of either intimate contact, or direct protoplasmic continuity, and may be mediated through the trophic activity of the nerve impulse (as in the case of the motor nerve and striated

muscle), or through the action of metabolites liberated on either side of the synapse.

2. The injured or moribund cell liberates toxins or metabolites which are injurious to the end-feet which terminate on it, and cause them to degenerate.

3. Since it is often more difficult to get prompt perfusion of the spinal cord after cutting ventral roots, the changes observed may be postmortem changes due to poor fixation.

As far as the first two possibilities are concerned, it is difficult to differentiate between them on the basis of the material and methods used here. In this material, there is no anatomic evidence of protoplasmic continuity at the synapse.

The third possibility we believe not to be a factor, for if the changes observed were due merely to poor fixation, they should be similar in all experiments, regardless of the time interval between operation and sacrifice. They are, as has been recorded, progressive in nature as the time interval lengthens, in that more end-feet become affected, and the number of end-feet on any one cell gradually decreases. Although the changes which occurred in response to chromatolysis were morphologically similar to postmortem changes, they are not necessarily due to the same cause. A degenerating end-foot may well degenerate in the same way, regardless of the cause of its morphological disintegration.

SUMMARY

1. With proper fixation (rapid penetration, minimum shrinkage and distortion) end-feet in the spinal cord of the cat may be stained with a variety of methods. The ones selected for use in these experiments were Cajal's silver impregnation method, and Mallory's connective tissue stain, following Regaud perfusion.

2. In the Cajal material of the normal cat spinal cord end-feet vary from small, clear round loops measuring 0.5 by $0.5\ \mu$ to large, elliptical, solid bodies, measuring about 3 by $5\ \mu$.

3. In Regaud fixed preparations end-feet do not vary greatly in size, and are round, granular, and solid, measuring about $1\ \mu$ in diameter. True loops are rarely seen.

4. In none of this material is there any evidence that all the end-feet are stained by either of these methods.

5. After hemisection of the spinal cord and cutting of dorsal roots, no variation from the normal was observed in end-feet below the level of the lesion.

6. Upon cutting ventral roots of the spinal cord, end-feet on the chromatolyzed anterior horn cells fragment and disappear in increasing numbers.

7. End-feet show rapid postmortem degenerative changes.

8. Because of the normal variability of the end-foot and the incompleteness of demonstration with the Cajal method, and because no significant differences from the normal can be seen after cutting the axon of which the end-foot is the termination, it is believed that the method is not reliable for following fiber tracts to their termination in the spinal cord.

9. The response of the end-foot to injury of its cell of termination is probably either on the basis of a chemical change in the cell, or interruption of trophic impulses, and not to direct protoplasmic continuity between the end-foot and the cell of termination.

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PLATE 1

EXPLANATION OF FIGURES

13 Anterior horn cell from side of the lesion in an animal sacrificed 4 days after section of ventral roots, illustrating eccentricity of the nucleus, and beginning neurofibrillar change. Remnants of fragmented end-feet can be seen, but the total number of remaining end-feet falls within the wide limits of normal variability. $\times 1080$.

14 Anterior horn cell from side of lesion in an animal sacrificed 7 days after ventral root section. The nucleus is pyknotic and the neurofibrillae appear pushed to the periphery of the cell body, leaving the perinuclear region more homogeneous. No fragmented end-feet can be seen, and the marked decrease in the total number of end-feet is striking. $\times 1080$.

